THE MOLTING HORMONES FROM THE EMBRYONATED EGG OF THE TOBACCO HORNWORM, MANDUCA SEXTA (L.)

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ABSTRACT

26-Hydroxyecdysone is the predominant molting hormone in 24- to 44-hour-old embryonated tobacco hornworm eggs, accounting for approximately 80% of the ecdysones present at this stage of development. This molting hormone was previously shown to be the major ecdysone present in 48- to 64-hour-old embryonated eggs of this insect. During both of these periods of embryonic development in the hornworm 20-hydroxyecdysone is a minor component, in contrast to its presence as the major ecdysone in the hornworm during certain stages of post-embryonic development.

In an earlier study, we reported that a new C_{27} molting hormone 26-hydroxyecdysone is the major ecdysone in 48- to 64-hour-old embryonated eggs of the tobacco hornworm, Manduca sexta (L.) (1). In addition to 26-hydroxyecdysone, three of the four remaining known naturally occurring C_{27} insect molting hormones, namely α-ecdysone, 20-hydroxyecdysone, and 20,26-dihydroxyecdysone, were also isolated but in considerably smaller amounts. Since 26-hydroxyecdysone accounted for about 90% of the total ecdysones that were taken from the egg, it was proposed that the major biosynthetic-metabolic pathway for the molting hormones during this stage of insect development involves the conversion of α-ecdysone to 26-hydroxyecdysone. We have continued our studies with the eggs of the tobacco hornworm to determine if the profile for the molting hormones in an earlier stage of embryonic development is similar to or different from that observed in the older embryonated eggs (1).

We accumulated for analysis approximately 4.6 kg (wet wt.) of 24- to 44-hour-old eggs. Although this mass is 0.4 kg less than that

accumulated in the former report, the percentage of eggs actually containing embryos (as estimated by determining the average hatch) in the samples of 24- to 44-hour-old eggs was 91% as opposed to 85% for the samples of 48- to 64-hour-old eggs (1). Since there was so little difference (<2%) in the number of embryonated eggs extracted the results obtained in this report may be considered directly comparable to those obtained in the previous study. The biological activity of extracts from 24- to 44-hour-old eggs determined by using the house fly assay (2) was found to be approximately two-thirds that found for the older eggs.

When we processed and separated the molting hormones, 26-hydroxyecdysone was again found to be the predominant molting hormone--accounting for nearly 80% of the total ecdysones isolated from 24- to 44-hour-old eggs. α-Ecdysone, 20-hydroxyecdysone, and 20,26-dihydroxyecdysone were also present, but as with the older eggs, in considerably lesser quantities relative to 26-hydroxyecdysone. Thus, the results are in keeping with our earlier report in which we proposed that the conversion of α-ecdysone to 26-hydroxyecdysone is a major step in the biosynthetic-metabolic pathway of the ecdysones during embryonic development in the tobacco hornworm (1). Of the four molting hormones taken from the egg, 20-hydroxyecdysone was the most difficult to detect and to isolate because of the relatively small amounts present. Although 20-hydroxyecdysone is the major molting hormone during certain stages of post-embryonic development in a number of species of insects (3, 4, 5) including the tobacco hornworm (6), it is but a minor component during both of the periods of embryonic development examined thus far in the hornworm (1). These findings with hornworm, and the recent report of a

C_{28} hexahydroxy molting hormone (Makisterone A) as the predominant ecdysone in the embryonated egg of the large milkweed bug Oncopeltus fasciatus (Dallas) (7), again emphasize the existence of different pathways for the biosynthesis and metabolism of steroid molting hormones (3, 5, 8) in different species of insects as well as in different stages of the same insect.

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were recorded at 60 Mc with a Varian A-60A NMR spectrometer (9) with deuterated pyridine as the solvent and TMS as an internal standard. The mass spectra were measured by using a LKB model 9000 gas chromatograph mass spectrometer (LKB Produkter, AB, Stockholm, Sweden); the samples were introduced directly into the ionization chamber, and the ionization energy was 70 ev. Silica Gel G plates were used for thin-layer chromatographic (TLC) analyses, and the solvent system was chloroform and ethanol (4:1) with wick (7).

Approximately 4.6 kg (wet wt.) of 24- to 44-hour-old embryonated tobacco hornworm eggs were extracted for their molting hormones according to procedures described elsewhere (1). The crude extracts (4.5 g) containing the ecdysones were chromatographed on silicic acid with increasing percentages of methanol in benzene (1, 7). The 90:10 benzene-methanol fraction (190 mg) that elutes mainly α-ecdysone, 20-hydroxyecdysone, and the 3-epi-20-hydroxyecdysone of the known naturally occurring C_{27} insect molting hormones was subjected to a 50 tube countercurrent distribution system of cyclohexane-butanol-water, 5:5:10, with 10 ml each of the upper and lower phases. The residue (30 mg) from countercurrent tubes 10-22 was purified by column chromatography on silicic acid, and Woelm alumina neutral grade I + 20% water (7) and by TLC to a mass of less than 200 μg of impure 20-hydroxyecdysone, R_f 0.15, M^+ 480.

The residue (37 mg) from countercurrent tubes 29-41 was purified by column chromatography on silicic acid and Woelm alumina neutral grade I + 20% water (7) and by thin-layer chromatography to a final mass of 1.0 mg of impure α-ecdysone, λ max 245 nm, (methanol), R_f 0.23, M^+ 464, methyl proton resonances at δ, 0.72 (18-H), 1.09 (19-H), 1.23, 1.31 (21-H), 1.41 (26- and 27-H).

The 75:25 benzene-methanol fraction (443 mg) from the silicic acid column that is known to elute 26-hydroxyecdysone and 20,26-dihydroxyecdysone (1) was further purified on Woelm alumina neutral grade I + 20% water (7). The methanol fraction (58 mg) from the alumina column was subjected to 50 transfers in a countercurrent distribution system as described for the 90:10 benzene-methanol fraction. The residue (10 mg)

from countercurrent tubes 1-6 was chromatographed on silicic acid to a final mass of 3.0 mg of substantially pure 20,26-dihydroxyecdysone, λ max 244 nm (methanol), R_f 0.05, the M^+ peak was not obtained; however, the compound gave a peak at m/e 478 (M-18), which indicated the loss of a molecule of water; methyl proton resonances at δ, 1.22 (18-H), 1.08 (19-H), 1.58 (21-H), 1.48 (27-H). The biological activities of the α-ecdysone, 20-hydroxyecdysone, and 20,26-dihydroxyecdysone isolated from embryonated eggs based on their purity were all equivalent to those of their corresponding authentic standards (6, 7, 10) using the house fly assay.

The residue (29 mg) from tubes 8-21 was purified by silicic acid to a final mass of 22 mg. Crystallization from ethyl acetate-methanol provided 5.3 mg of needle like crystals, mp 259-261°, λ max 245 nm (methanol) ε 12,100, R_f 0.08, countercurrent distribution coefficient (K value) 0.39 (1, 7), M^+ 480, methyl proton resonances at δ, 0.74 (18-H), 1.08 (19-H), 1.23, 1.32 (21-H), 1.47 (27-H). The biological activity of the 26-hydroxyecdysone from the egg was as previously reported (1) for this compound using the house fly assay.

The four molting hormones isolated from 24- to 44-hour-old embryonated eggs were found to be identical to their corresponding authentic standards by TLC and NMR and mass spectral analyses.

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TRIVIAL AND IUPAC EQUIVALENT NAMES

α-Ecdysone = 2β,3β,14α,22R,25-Pentahydroxy-5β-cholest-7-en-6-one
20-Hydroxyecdysone = 2β,3β,14α,20R,22R,25-Hexahydroxy-5β-cholest-7-en-6-one
26-Hydroxyecdysone = 2β,3β,14α,22R,25,26-Hexahydroxy-5β-cholest-7-en-6-one
20,26-Dihydroxyecdysone = 2β,3β,14α,20R,22R,25,26-Heptahydroxy-5β-cholest-7-en-6-one
Makisterone A = 2β,3β,14α,20,22,25-Hexahydroxy-24ξ-methyl-5β-cholest-5β-cholest-7-en-6-one
3-Epi-20-hydroxyecdysone = 2β,3α,14α,20R,22R,25-Hexahydroxy-5β-cholest-7-en-6-one